**"TO BE DETERMINED" TOPICS on the Mycoplasma Guideline**
**For the May 2001 meeting in Tokyo (March 2001 draft)**
**Topics in order of discussion, not order in guideline**

**1.** Section **2.1 SAMPLES,** has yet to be discussed at a Mycoplasma VICH working group meeting. The paragraph, **2.1 SAMPLES**, included in the (h) draft of the guideline (and below) is proposed by the topic leader after considering the responses received in completion of the attached table below.

**2.1. Samples**

(TO BE DETERMINED) Tests for Mycoplasma contamination shall be done on final container samples of each serial, lot, or batch of cell culture or chicken embryonated egg origin live vaccine. Pooled or single batch bulk harvest stages must be tested instead of the final product for killed vaccine and may be tested instead of final product for live vaccines. Each lot of master seed, primary and master cell stock, working seed, and ingredients of animal origin shall be tested for Mycoplasma contamination.

**Segments of the Vaccine Production Process Required by Regulations to be Tested for Mycoplasma Contamination: By Country and Test Method**

| Country Test Method | Ingred. Animal Origin | Master Seed Virus | Master Cell Stock | Working Cell Pass. | Working Virus Stocks | Bulk Harvest | Final Product |
|---|---|---|---|---|---|---|---|
| Europe Culture | X | X | X | X | | X | X[1] |
| Europe DNA Stain | X[2] | X | X | X | | | |
| Japan Culture | | X[1] | | | | X[2] | X[2] |
| Japan DNA Stain | | | | | | | |
| Canada Culture | X[a] | X | X | X[b] | X[b] | X[c] | X[c] |
| Canada DNA Stain | | X | X | X[b] | X[b] | | |
| Australia & New Zealand Culture | X | X | X | | | X | X |
| Australia & New Zealand DNA Stain | | | | | | | |
| United States Culture | X[1] | X | X | | | X | X[2] |
| United States DNA Stain | | | | | | | |

US[1] - If material is not to be heat-sterilized.

US[2] - Harvests, harvest pools OR final container samples must be tested. For killed products, applies only if killing agent is not proven to kill mycoplasma.

Ph.Eur[1]- Final product testing does not need to be performed if bulk harvests have been tested.

Ph.Eur[2]- Optional may be used for media screening.

J[1]- The test for this process is carried out for only the products, the methods for production of those master seed lots are prescribed precisely, e.g. Hog Cholera Live Products.

J[2]- The tests for these processes are not carried out for killed products.

CAN[a]- If material is not to be heat-sterilized.

CAN[b]- In certain casesworking cells or viral stocks can be tested in place of master stocks if little or no master stocks remain or where master and working are one and the same.

CAN[c]- Harvests, harvest pools, OR final container samples must be tested. For killed products, applies only if killing agent is not proven to kill Mycoplasma.

**2.** Determine a process by which a laboratory's proficiency in Mycoplasma detection can be demonstrated utilizing those standard reference strains being produced by the European Department of the Quality of Medicines (EDQM). The following process was proposed by the topic leader in the draft circulated after the VICH meeting in Ames in July of 2000. Should the explanation of the production and utilization of these standard reference strains (**Appendices 3.2**) be left in the Appendices of the guideline? Are there any changes that need to be made to the validation process that have become known because of the European experience with these references or because of import restrictions in Japan, US, Australia, Canada, or other interested countries?

### 3.2 REFERENCE PRODUCTION AND UTILIZATION

The Master references of the 5 strains of Mycoplasma listed in **Section 2.3.1** will be isolated by labs of the European Union and donated to the European Department of the Quality of Medicines (EDQM). EDQM will produce a sufficient quantity of these Master references to be distributed to the 3 regional government laboratories (Japan, EU, and USA) of this VICH Mycoplasma working group. The regional government labs will then distribute these master references to those labs in their region wishing to be validated in Mycoplasma testing. The producing European laboratories will make the references so that they contain approximately 100 CFU (70-130 CFU) per specified inoculum. A group of laboratories in the three regions will standardize these references and validate the CFUs.

For Mycoplasma test validation each laboratory will be sent 3 vials of each reference strain depending on the types of products being tested. A different production lot of the mediium or media shall be used for each vial of a reference strain. After the laboratory completes the testing they shall report their results to the regional government laboratory supplying the reference vials. At the time of the validation testing each laboratory shall produce and validate working references from the Master references. These working

references shall be used to test subsequent batches of Mycoplasma media used in the labs testing for Mycoplasma contamination in veterinary biologics.

**3.** Determine if direct inoculation of an agar plate, **Section 2.3.5.1,** on day 0 should be included in the guideline**.** Only one additional comment was received in addition to the data presented by the USDA at the July meeting. Dr. Mike Edgington of an independent Mycoplasma testing lab, Microsafe, indicated that in the cases of heavily contaminated samples a more timely result helps with contamination within a production facility.

> **2.3.5.1** Inoculate each plate of each solid medium with 0.2 ml of product to be examined and inoculate 10 ml per at least 100 ml of each liquid medium. Incubate the agar plates at $35^0$C to $38^0$C, microaerophilically, for 10-14 days in an atmosphere of adequate humidity to prevent desiccation of the surface. Incubate the liquid media at $35^0$ C to $38^0$ C in air or microaerophilic conditions for 21 days. At the same time incubate . . .

**4.** Determine if liquid medium needs to be incubated microaerophilically or if air incubation is comparable**.** Again Dr. Mike Edgington of Microsafe was the only person responding to this change. Microsafe's experience indicated that the conditions chosen for incubation, air vs. microaerophilic, do not influence the growth of mycoplasmas to any significant degree. A point of caution, liquid medium incubated in an enriched $CO_2$ should have the top of the container firmly closed, as an ingress of $CO_2$ can lead to acidification of the medium and a false positive color change.

**5.** Determine if **Section 3.4 Inhibitory Substances** should be changed to include a "notable reduction" rather than "absent" growth of the working references. It has been suggested that a definition of a "notable reduction" could be a delay in growth between the working reference control and the test substance of not more than one passage. For example, if the working reference control sub-culture shows growth on the plates at day 3 of incubation then the test substance culture must show growth at least by the day 7 plates to be considered free of inhibitory substances.

**6.** Determine what is meant by the 1 ml subculture in **Section 2.4.2.2.** The USDA has tried this method and has concluded that the sensitivity of this method is influenced by how this step in the procedure is performed. Does this mean a 1 ml subculture of the: supernate, trypsinized cells or the dilution of the trypsinized cells that will give 50% confluence at 3-5 days? How should this section be reworded to alleviate this confusion?

**7.** Determine how the guideline should reference the PCR technique for the detection of mycoplasma contamination. Dr. Itoh suggested the WG not include the procedure in the guideline because it was not an official regulatory test in any of the three regions involved in the VICH process. The topic leader suggests that the WG add the following sentences to the end of **Section 1.4 General principles**; "The use of polymerase chain reaction currently is not sufficiently developed for product, starting, or in-process material mycoplasma contamination testing. However, its use is encouraged in parrallel testing and isolate identification to further

develop and refine the technique." The WG should decide whether to include specific possible or recommended techniques or references to techniques in the guideline.

**8.** Determine if **Section 2.3.3** should be require the testing laboratory to change reference species from test session to session as a better control on the growth promotion qualities of the broth and agar.

**9.** Determine if the staining of mycoplasma colonies on agar should be required in **Section 2.3.6** to confirm contamination.